STUDIES ON THE TREMATODES OF WOODS HOLE. III. THE LIFE CYCLE OF MONORCHEIDES CUMINGIAE (MARTIN) WITH SPECIAL REFERENCE TO ITS EFFECT ON THE INVERTEBRATE HOST

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This work was done during the several past summers spent at the Marine Biological Laboratory. A synopsis of this research was given before the 1939 Columbus, Ohio meeting of the American Society of Parasitologists. *Monorcheides cumingiae* parasitizes the intestines of flounders and eels, two important food fishes, and so may be of some economic importance. Very little work, particularly of an experimental nature, has been done on the life cycles of the members of the family *Monorchidae* to which this species belongs.

The family name *Monorchidae* was erected by Odhner (1911) with *Monorchis monorchis* (Stossich) as the type genus and species. As the family name indicates, one testis is characteristic of the group, but the genus *Monorcheides* is an exception in that two testes are present. The genus *Monorcheides* was created by Odhner (1905) for some small worms found in the intestine of the marine fish, *Lumprenus medius*, collected along the west coast of Spitzbergen.

Although the members of this family are rather widely distributed. very little work has been done to trace the biological relationships of the stages in the life cycles. According to Yamaguti (1938), Nagano (1930) found that a cercariaeum that developed in rediae and encysted in *Bulimus striatus japonicus* (Pilsbury) became the adult of *Asymphylodora tincae*. Yamaguti (1934) states that the species Nagano was working with was probably *A. macrostoma* Ozaki but in a later paper (1938) refers this form to *A. japonica* Yamaguti. Yamaguti (1934) reported finding what he believed to be the larval form of *A. macrostoma* Ozaki encysted in the peribuccal connective tissue of *Chaenogobius macrostomus*, although no feeding experiments were conducted. In 1938 this same author reports that on the basis of anatomical and ecological evidence a cercariaeum and its cyst found in *Bulimus striatus japonicus* (Pilsbury) probably represent stages in the life cycle of *Asymphylodora japonica* Yamaguti. In the same paper he

reported finding the larva of *Asymphylodora macrostoma* Ozaki encysted in the peribuccal connective tissue and gill arches of *Gnathopogon elongatus caerulescens* (Sauvage) from Lake Biwa and from *Cobitus biwae* Jordan and Snyder from the Katura River, Japan. During the past summer it was possible to demonstrate that *Cercaria cumingiae* Martin (1938), when fed under experimental conditions to flounders and eels, develops into an adult belonging to the genus *Monorcheides*.

In addition to the genus *Monorcheides*, the family *Monorchidae* includes species belonging to the following genera which have been collected from widely scattered regions: *Asymphylodora* Looss 1899 from Egyptian (Looss, 1899), Japanese (Yamaguti, 1938), and Indian (Srivastava, 1939) waters; *Bivesicula* Yamaguti 1934 from Japan; *Genolopa* Linton 1910 from Bermuda and Dry Tortugas (Linton, 1910), Beaufort, North Carolina (Manter, 1931), and North Queensland (Nicoll, 1915); *Hurleytrema* Srivastava 1939 from India; *Lasiotocus* Looss in manuscript and *Pristisomum* Looss in manuscript (Odhner, 1911) from Mediterranean and British (Nicoll, 1915) waters; *Monorchis* Looss 1902 from the Mediterranean Sea and British waters (Nicoll, 1915); *Paramonorcheides* Yamaguti 1938 from Japan and from the Galapagos Islands (Manter, 1940); *Paraproctotrema* Yamaguti 1934 from Japan; *Physochoerus* Poche 1925 from the Mediterranean Sea; *Proctotrema* Odhner 1911 from the Scandinavian arctic (Odhner, 1911), from Beaufort, North Carolina, Costa Rica, and the Galapagos Islands (Manter, 1931, 1940), from Japan (Yamaguti, 1934) and from the Karachi and Arabian Seas (Srivastava, 1939); *Proctotrematoides* Yamaguti 1938 from Japan; and *Telolecithus* Lloyd and Guberlet 1932 from Puget Sound and from Panama (Manter, 1940).

MATERIALS AND METHODS

Living material, preserved whole mounts, and serial sections were used in this study. Living material is particularly useful in working out the finer details of the anatomy of the larval stages. Neutral red was used to stain the living material, while on preserved specimens Mayer's paracarmine was used on whole mounts and Delafield's hematoxylin and eosin were used on sectioned material. The mounting medium, "Clarite," was used and proved very satisfactory.

The mollusks, *Cumingia tellinoides*, were placed in finger bowls and daily observations were made to detect the presence of emerging cercariae. Infected clams were isolated individually and heavy infections of metacercariae were established in them. Heavy infections were produced also in clams that were not infected with sporocysts. In other words, some clams that had not served as first inter-

mediate hosts were used as second intermediate hosts.   Infected clams were fed to flounders and eels that had been kept in aquaria for several weeks to allow the maturation of any worms that might have been present in the intestines of these fishes when they were brought into the laboratory.

OBSERVATIONS

The life cycle of *Monorcheides cumingiae* involves the development of sporocysts, cercariae, and metacercariae in the marine bivalve, *Cumingia tellinoides* (Conrad), and the development of the adult, under experimental conditions, in the intestines of eels and flounders.

THE LIFE CYCLE

The Sporocyst

The sporocysts are simple sac-shaped structures that develop in the visceral mass of the clam.   Because of the large number of sporocysts within a single clam it seems probable that there is a daughter sporocyst generation although this has never been observed.   The entire visceral region may be riddled with sporocysts, resulting in partial or complete castration and other disturbances.   The intestine was usually surrounded by sporocysts and it is likely that this is the avenue of entrance of the miracidia.   The fact that the eggs of this parasite failed to develop when kept for over three weeks in sea water suggests that they must be eaten by the clam in order for development to take place.   The wall of the sporocyst is very thin and contains scattered nuclei.   However, at the ends of the sporocysts thickened knobs of cells are present and they seem to take part in the formation of germ balls.   The nuclei in these knobs enter protoplasmic extensions into the lumen of the sporocyst and, finally, actually leave the cytoplasm behind and take part in cercarial formation.   The lumina of the sporocysts contain fragments, probably cellular, which stain with eosin.

The Cercaria

The cercaria bears two conspicuous eyespots in the anterior half of the body which still are evident in the recently encysted metacercaria (Fig. 5).   The oral and ventral suckers are approximately equal in size.   The excretory system is relatively simple and is represented by the formula $2[(2 + 2) + (2 + 2)]$.   A mass of cells directly behind the ventral sucker represents the anlage of the reproductive system.   The cercariae emerge from the clam by way of the excurrent siphon and swim about immediately.   The light intensity is a controlling factor of the direction of swimming because the cercariae show a definite

negative response to light. Eventually the cercariae come in contact with the tissues of the clam, either by being forcibly taken in by the incurrent siphon or by coming in contact with the mantle or foot through their own swimming efforts. In both cases they soon shed their tails and work their way into the clam tissue where they encyst. Apparently, encystment does not take place prior to a period of swimming, i.e. the cercariae do not encyst in the time interval between emerging from the sporocysts and leaving the clam. The factor, or factors, controlling the time of encystment are unknown. The fact that a swimming period must occur before encystment takes place is of definite advantage to the parasite in increasing its range, since clams other than the infected one may become second intermediate hosts. In this connection it was found that another small clam, *Tellina tenera* Verrill, could serve as a second intermediate host. This same species may very rarely serve as the first intermediate host since one of several hundred examined was infected with sporocysts.

In the original description of *Cercaria cumingiae* Martin 1938, it was postulated that this species probably belonged to the family *Allocreadiidae* because of anatomical similarities to some of the cercariae of this family. However, the experimental results have disproved this postulation. Nevertheless, these similarities suggest a possible relationship of the family *Monorchidae* to the family *Allocreadiidae*.

EXPLANATION OF PLATES

Abbreviations used:

C . . . . .cirrus		*OV* . . . . . . .ovary	
CS . . . . . . .cirrus sac		*P* . . . . .pharynx	
E . . . . .eyespot		*PR* . . . . . . .prostate tissue	
EB . . . . . . .excretory bladder		*S* . . . . .seminal vesicle	
EG . . . . . . .egg		*SP* . . . . . . .spine	
G . . . . .genital pore		*T* . . . . .testes	
I . . . . .intestine		*U* . . . . .uterus	
M . . . . . . .metacercariae		*V* . . . . .vagina	
O . . . . .oral sucker		*VI* . . . . . . .vitellaria	

DESCRIPTION OF PLATE I [1]

Fig. 1. Longitudinal section through the siphon of *Cumingia tellinoides* showing metacercariae just inside the muscular layer of the siphon.

Fig. 2. Section through the gill and part of the body of *Cumingia tellinoides* showing metacercariae in the gill and sporocysts (*a*) in the body.

[1] I wish to express my appreciation to Dr. E. J. Kohl of Purdue University for making the photomicrographs in Plates I and II.

Plate I

The Metacercaria

Upon shedding its tail and encysting the cercaria becomes a metacercaria. During the metacercarial stage certain important changes occur: the eyespots start to disintegrate, the digestive system becomes much better developed with the ceca reaching to near the posterior end of the body, there may be a slight increase in the number of cephalic glands, the excretory bladder loses its thick-walled appearance and becomes a thin sac due to the breaking down of the cells of the wall, there is an increase of two or three times in the size of the body, and the cuticula increases in thickness. A very interesting change occurs in the staining reaction of the nuclei with the aging of the metacercariae. The nuclei of recently encysted metacercariae can hardly be distinguished but as the metacercariae become older the nuclei stain heavily with Delafield's hematoxylin (Fig. 5). The significance of this change is unknown. During the metacercarial stage there is very little differentiation of the genital region.
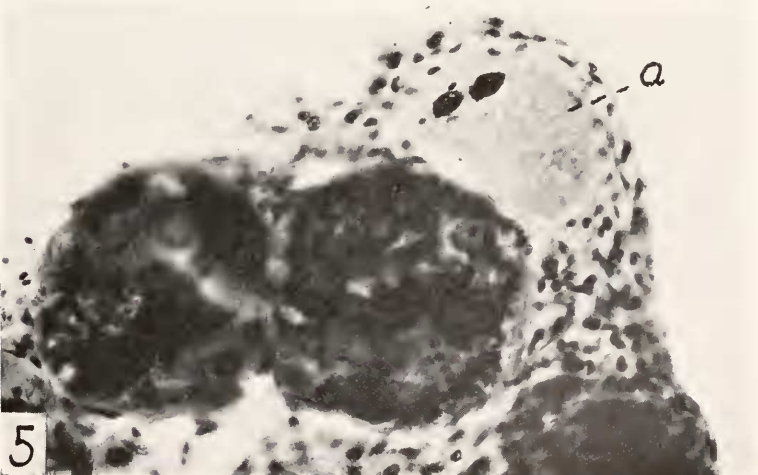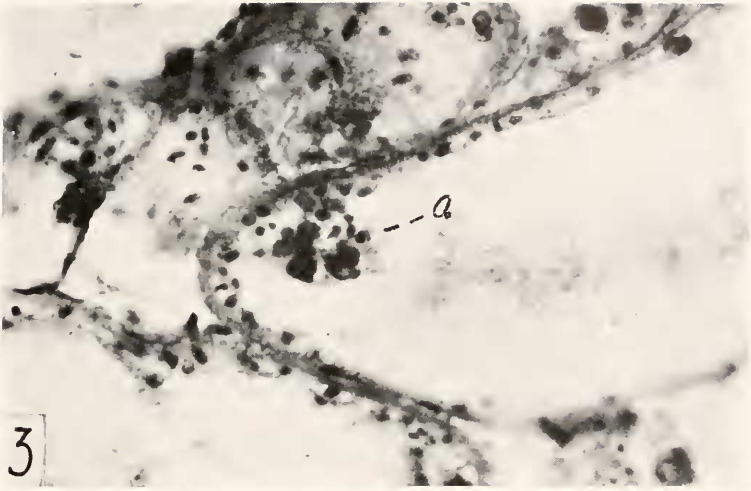
The Adult (Figs. 6 and 7)

Under natural conditions, it seems probable that the definitive fish host obtains its infection by eating the siphons or the entire clam infected with metacercariae. *Cumingia tellinoides* and *Tellina tenera* extend their siphons a considerable distance beyond the edge of the shell and these structures could attract the attention of fishes. In the laboratory, small puffers, *Sphaeroides maculatus* (Schneider), were observed to bite off the siphons of *Cumingia* and other clams. In tracing this life cycle, entire infected *Cumingia* were removed from their shells and fed to three flounders and to three eels. Six of each species of fish were reserved as controls. With the exception of the infected *Cumingia*, both experimental and control fishes received the same type of food, namely clam meat, *Venus mercenaria*, *Mya arenaria*, *Modiolus modiolus*, and *Mytilus edulis*, which was examined carefully for any possible trematode infection. One of the eels, however, was
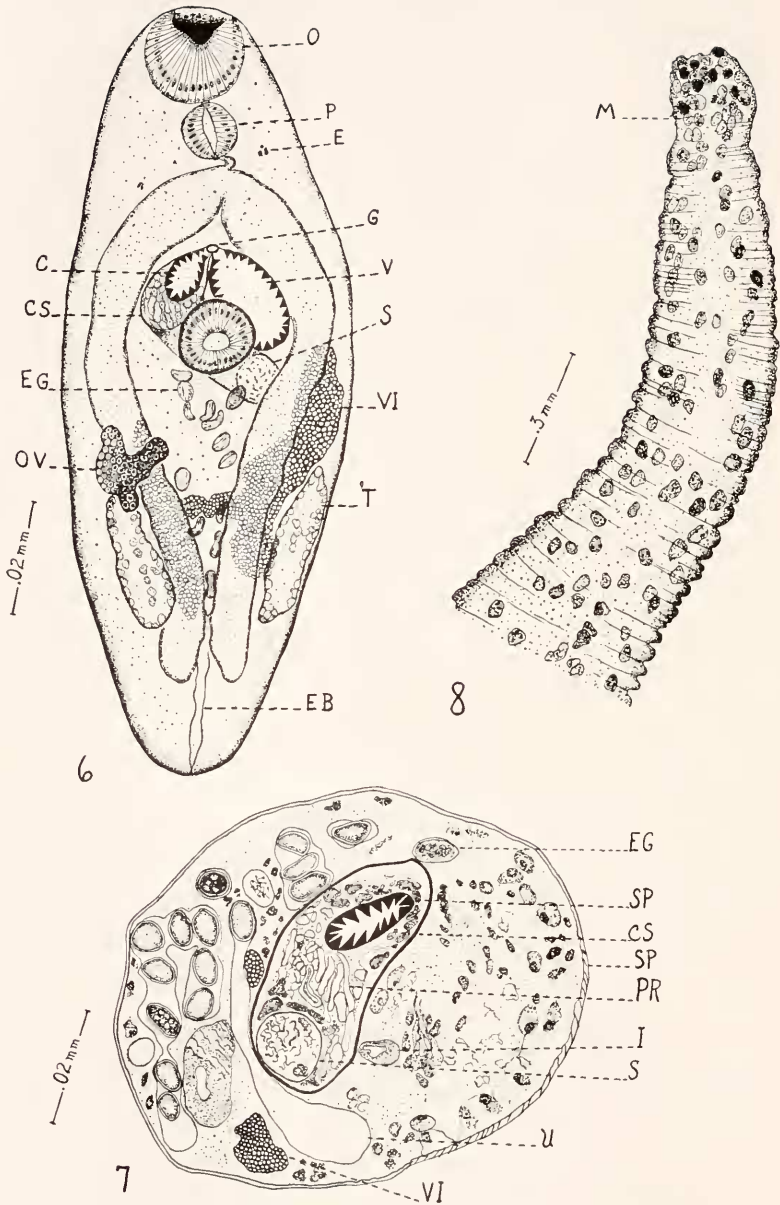
PLATE II

6

8

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DESCRIPTION OF PLATE III

FIG. 6.   Adult *Monorcheides cumingiae.*
FIG. 7.   Transverse section of an adult *Monorcheides cumingiae.*
FIG. 8.   Whole mount of a siphon of *Cumingia tellinoides* showing the distribution of metacercariae.

fed only infected *Cumingia* and after four weeks of feeding it was found to have an infection of over one hundred *Monorcheides cumingiae* in various stages of development.   In all, four of the six fishes experimentally fed infected *Cumingia* were found to harbor *Monorcheides cumingiae* when examined four weeks after the initial feeding.   In both the eels and the flounders, the parasites were found in the mucus of the anterior portion of the intestine.   No ulceration of the intestine was observed.   The controls were negative.

The adults are extremely small trematodes as shown by the following measurements based on five specimens, all of which contained eggs. The body length is 0.255–0.318, with an average of 0.30 mm.; the body width is 0.1–0.164, with an average of 0.142 mm.   The cuticula is spined with the spines being somewhat more conspicuous on the anterior half of the body.   The oral sucker is terminal or subterminal and measures from 0.0315–0.0415, with an average of 0.0367 mm. in length by 0.0348–0.0498, with an average of 0.0408 mm. in width.   The ventral sucker measures from 0.0232–0.0332, with an average of 0.026 mm. in length by from 0.0291–0.0332, with an average of 0.032 mm. in width.   The very short prepharynx (about 0.003–0.004 mm. in length) is followed by a pharynx that is on the average 0.0185 mm. in length by 0.022 mm. in width.   The esophagus is approximately 0.0124 mm. long and it leads to the forking of the intestine which occurs about midway between the two suckers.   The intestinal rami approach, but do not extend to, the posterior end of the body.   The reproductive systems are well developed and both the cirrus and vagina are armed with relatively large triangular spines.   The genital pore is located in the mid-ventral region just posterior to the forking of the intestine.   The cirrus sac is elongate and extends from the genital pore to a short distance posterior to the ventral sucker.   It encloses a seminal vesicle, numerous "prostate" cells, and a spined cirrus.   The two testes are laterally situated about half-way between the ventral sucker and the posterior end of the body.   They are composed of loosely arranged tissue.   The single ovary is located just above the testis on the right side of the body.   Its margin is irregularly lobate. No seminal receptacle was observed.   The coils of the uterus may fill the posterior half of the body, particularly in the region between the testes.   The uterus terminates anteriorly at approximately the middle of the median surface of the vagina.   The vagina is relatively thickwalled, and is spined throughout its entire length.   It is quite large, measuring approximately 0.0224 mm. wide by 0.0332 mm. long.   It generally lies on the left side of the body.   The spines arming the vagina are similar in shape and size to those found in the cirrus.   The

eggs are amber-colored, oval in outline, operculate, and measure about 0.016 mm. long by 0.011 mm. wide.   Mature eggs were removed from the uteri of several worms and were kept for over three weeks under conditions favorable for hatching but very little change took place in them.   This suggests that the eggs must be eaten by the clam host before development will proceed.

The adult *Monorcheides cumingiae* differs from the other members of the genus in the following ways.   The size of the body, suckers, pharynx, and eggs is smaller in *M. cumingiae* than in any other member of the genus.   The shape of the vaginal and cirrus spines is triangular in *M. cumingiae* but in the other species, judging from figures, they are much more elongate.   The spines of the cirrus of *M. cumingiae* are uniform in size while in *M. diplorchis* Odhner the spines on the median side are longer than those found on the lateral side of the cirrus.   The vitellaria of *M. soldatovi* Issaitschikow extend from the posterior border of the pharynx to the level of the middle of the ovary, in *M. diplorchis* the yolk glands extend from the level of the forking of the intestine to a short distance posterior to the anterior margin of the ovary, while in *M. cumingiae* these glands may extend from the posterior margin of the ventral sucker to the anterior margin of the testes. *Monorcheides* (?) *petrowi* Layman probably does not belong to this genus at all because the ovary is posterior instead of anterior to the testes.   The genus *Paramonorcheides* Yamaguti is closely related, if not synonymous, with the genus *Monorcheides*.   The principal difference between the two seems to be a sac-shaped excretory bladder in the former and a Y-shaped one in the latter.   Some specimens of *Monorcheides cumingiae* give the appearance of having a Y-shaped bladder due to the distention of the lower portions of the main collecting ducts.   It is likely that Odhner believed that these enlarged collecting ducts were continuations and part of the bladder.

Nagano (1930), according to Yamaguti (1938), has shown that the larval form of *Asymphylodora tincae* is a tailless cercaria, or the so-called cercariaeum, lacking eyespots.   It is apparent, therefore, that there are at least two larval types in the family.   The larval form of the genus *Bivesicula* Yamaguti 1934 is evidently similar to the cercaria of *Monorcheides cumingiae* in the possession of eyespots since remnants of these structures are found in the adults.

Effect of Parasite on Clam

The effects of the parasite on the clam may be divided into two categories, first the damage done by the sporocysts when the clam serves as the first intermediate host, and second the injury caused by

the cercariae when they re-enter to become metacercariae or when the clam is serving as the second intermediate host.   Of the two, the first undoubtedly is more extensive since most of the visceral region may become filled with sporocysts.   The sporocysts seem to be more numerous along the wall of the gut and along the periphery of the body in lighter infections but in severe infections they fill the tissues between these two regions.   The fact that the sporocysts first appear along the wall of the gut seems to support the evidence obtained from the unsuccessful efforts to hatch the eggs of this worm, that the eggs are eaten by the clam and hatch in the gut, infection taking place through the wall of the digestive tract.   The normal tissue seems to be pushed aside and crowded upon itself until disintegration takes place.   Here and there between sporocysts portions of host tissue may remain in an apparently normal condition.   Just how this replacement of host tissue by the parasite occurs is unknown.   Perhaps some of it is by an enzymatic dissolution of the host cells or perhaps it is by the compression of the host tissue by the parasite.   If the latter is true, and there is some evidence to support this contention because here and there between the sporocysts distorted host cells can be found, the parasite tissue must be able to exert a greater growth pressure than the host tissue.   The fact that host tissue may remain in certain regions entirely surrounded by sporocysts seems to indicate that the action is not an enzymatic one, or if it is, some of the host's cells are more resistant than others to this action.

The effects of the penetration of the cercariae are confined for the most part to those tissues of the clam that are in contact with the water, such as the siphons, mantle, foot, and gills.   The siphon tissue, particularly that region next to the lumen and just below the muscular layer, is a favorite one for the penetration of the cercariae (see Fig. 1). Eighty metacercariae were counted in one longitudinal section of a siphon.   The distal end of the siphon may become so filled with metacercariae that it presents a frayed appearance.   Frequently the heavily infected siphon is autotomized.   Although the incurrent siphon generally contains a larger number of metacercariae, the excurrent siphon may become rather heavily infected.   Some encystment takes place in the mantle but probably because of its thinness it is incapable of supporting many metacercariae.   The muscle of the foot of the clam becomes very heavily infected.   Between ninety and one hundred metacercariae were observed in one longitudinal section of the foot.   The metacercariae in this region seem to induce the formation of a deeply staining (with Delafield's hematoxylin) deposit which may be an attempt by the clam to protect itself.   It, however, is not

a very effective protective device because recently encysted meta-cercariae were found in tissue that also contained older metacercariae. The mantle also may form small amounts of this deposit, in some cases even when there are no metacercariae present in that particular region. Therefore, it is likely that the deposition of this material is not a specific response to the action of the parasite but, nevertheless, the amount of the material deposited may be increased by the presence of the metacercariae. With the relatively large amount of surface exposed it seems rather remarkable that so few metacercariae are found in the visceral region. Perhaps the presence of sporocyst tissue may act as a barrier to the entrance of cercariae, or, it may be that the visceral tissue does not have sufficient rigidity for the penetration of cercariae for metacercariae are found in regions of the body where muscle fibers occur near the surface. The cercariae frequently penetrate the gill filaments forcing the thin membranes apart so that they surround the metacercariae. Some of the more heavily infected filaments are distorted considerably.

The damage done by the parasite to the invertebrate host is undoubtedly much more extensive than that received by the vertebrate host. However, in spite of the very extensive damage suffered by the clams they lived for several weeks, even without food except for the small amount that would be obtained from the daily changes of sea water.

Summary

1. The life cycle of *Monorcheides cumingiae* (Martin) involves the development of sporocysts, cercariae, and metacercariae in the marine bivalve, *Cumingia tellinoides*, and the development of the adult, under experimental conditions, in the flounder and eel.

2. Another clam, *Tellina tenera*, can serve as an alternative second intermediate host and on one occasion was found to act in the capacity of a first intermediate host.

3. Attempts at hatching the eggs of this trematode failed. Therefore it seems probable that the eggs must be eaten by the clam before further development will take place.

4. In spite of the extremely heavy infections, the clams survived for several weeks with no more food than would be obtained from the daily changes of sea water.

5. A free-swimming period is apparently necessary before the cercariae will encyst. This is an advantage to the parasite in increasing its range.

6. The cercariae encyst in large numbers in the siphons, particularly the incurrent, and foot. Metacercariae are found in smaller numbers in the gills, mantle, and, very rarely, in the visceral region.

7. There is a marked change in the staining reaction of the nuclei of recently encysted and older metacercariae. When both receive the same treatment, the nuclei of the young metacercariae take up little or no Delafield's hematoxylin while the nuclei of the older metacercariae are heavily stained.

8. Although the metacercariae seem to induce the surrounding clam tissues, particularly in the foot, to increase the deposition of a darkly staining material, this substance does not inhibit super infection.

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